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ORIGINAL ARTICLE BRIEF REPORT

Human Infection Due to Recombinant Vaccinia–Rabies Glycoprotein Virus

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Introduction



RABIES IS A FATAL VIRAL DISEASE TRANSMITTED FROM ANIMALS TO HUMANS. IT causes more than 35,000 human deaths per year.¹ Successful application of veterinary vaccines can eliminate canine rabies in an area, but control of rabies in free-ranging carnivores requires other strategies, such as oral vaccination.² Live viral vaccines containing modified live rabies or recombinant vaccinia–rabies glycoprotein virus, placed in a bait, are used for disease control in Europe and North America.²⁻⁵ In the United States, more than 22 million doses of vaccinia–rabies glycoprotein vaccine were distributed from 1990 to 2000, mainly to control rabies in raccoons in the eastern states and in foxes and coyotes in Texas.⁶⁻⁸ Despite contact with bait containing vaccinia–rabies glycoprotein vaccine by nontarget species, including

domestic animals and humans, no substantial adverse health effects of the vaccine were reported, and no lesions related to infection with vaccinia–rabies glycoprotein virus were detected in a wide variety of immunocompetent species.^{9,10} We have now documented an infection with the vaccinia–rabies glycoprotein virus after transdermal exposure in a woman with a chronic skin condition.

Case Report

Figure 1.



Pustules and Localized Erythema on the Medial Forearm of the Patient.

During September 2000, a 28-year-old woman from northeastern Ohio who had epidermolytic hyperkeratosis¹¹ and was 15 weeks pregnant presented to her physician with lesions that had appeared after she was bitten by her dog six days earlier. She had sustained mild abrasions on her forearm and a puncture wound on a finger. The finger bled and was washed thoroughly with soap and water. She did not notice bleeding on the superficial forearm abrasion and did not wash it. Three days after the bite, she observed two small blisters approximately 6 cm apart on her forearm, which progressed to nontender, vesicular lesions that were approximately 1.5 cm in diameter at the time of her initial presentation. She was given amoxicillin–clavulanate. Two days later, she presented to a hospital emergency room with progressive pain, erythema, swelling of the left forearm, and necrosis of the vesicular areas, which had expanded to 2 cm in diameter ([Figure 1](#)).

The patient was admitted with a diagnosis of cellulitis and was given intravenous cefazolin. The patient was afebrile, with a white-cell count of 9700 per cubic millimeter and an erythrocyte sedimentation rate of 56 mm per hour. The next day, treatment was switched to ampicillin–

sulbactam. During history taking, the patient revealed that she had sustained her wounds while removing a bait containing vaccinia–rabies glycoprotein virus vaccine, intended for raccoons, from her dog's mouth. She had not been vaccinated against either smallpox or rabies. By the third day of hospitalization, swelling and erythema worsened, left axillary adenopathy was present, and the necrotic lesions were larger. She was taken to surgery for incision and drainage of a presumed abscess in the left forearm 10 days after the dog bite. Two necrotic scabs were removed, and a compartment fasciotomy was performed. There was only scant pus and no loculations. Bacterial cultures were obtained and intravenous antibiotics were continued. Serum and swabs of the affected area were sent to the Centers for Disease Control and Prevention (CDC).

The next day, the patient's arm appeared less swollen and erythematous, and axillary tenderness improved. Amoxicillin–clavulanate was prescribed, and she was discharged on the following day. Two days later, she had worsening erythroderma that was thought to be a reaction to the antibiotics, and treatment with amoxicillin–clavulanate was discontinued. The next day, she presented to the hospital emergency room with general erythroderma, mild burning sensations and a feeling of tightness in the face, and generalized exfoliation on her face and neck. Her incision was healing, and she felt better overall. Five days later, a thick layer of epidermis exfoliated from her palms and soles. Ten days later (30 days after the dog bite), a residual eschar was removed for laboratory examination. Exfoliation had stopped by four days later, and her wounds had healed. The patient remained free of symptoms; her pregnancy was normal, and she delivered a healthy child in March 2001. Samples from the placenta and serum from umbilical-cord blood were shipped to the CDC.

Methods

Table 1.

TABLE 1. ANALYSIS OF POLYMERASE-CHAIN-REACTION PRODUCTS.*				
PRIMER	SENSE	SEQUENCE	GENE TARGET	LOCATION OF SEQUENCE
VS3	Forward	5'GGTCCCTATTGTTACAGATGGAAGGG3'	Thymidine kinase	8–33
VA3	Reverse	5'GTCCCCATCGAGTCGGCTAC3'	Thymidine kinase	540–559
GS3	Forward	5'CCACACCAAGATGATGTAGAGCCCGTAF	Glycoprotein	329–357
GA3	Reverse	5'AAACAAGGTGCTCAATTTCCTTGCGGAA3'	Glycoprotein	852–882

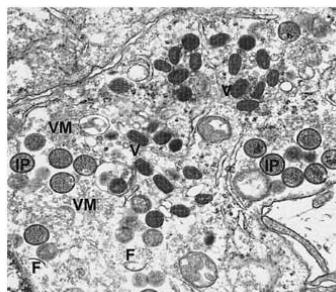
*The primers listed were used for the polymerase chain reaction (PCR) or sequencing. The location of each nucleotide sequence was determined by comparison with the published sequences of the gene targets. The sequence of the 100 percent homologous vaccinia–rabies glycoprotein gene and vaccinia virus glycoprotein messenger RNA (M38452) in GenBank.^{11,12} Sequence data were obtained with use of the ABI 377 automated sequencer and the ABI Prism Big Dye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems). Sequencing analysis of the polymerase-chain-reaction products was performed with the above primers. Sequence analysis of the samples from the patient demonstrated 100 percent homology with the vaccinia–rabies glycoprotein virus samples (550 bp with the use of the VS3 primer for the vaccinia–rabies kinase gene and 329 bp with the use of the GA3 primer for the rabies virus glycoprotein gene).

Analysis of Polymerase-Chain-Reaction Products.

After the patient gave informed consent, material from the swabs and homogenized portions of the eschar were inoculated onto a monolayer of Vero cells and maintained at 37°C. Approximately 48 hours after infection, cells scraped from a tissue-culture flask underwent centrifugation, and the pellet was processed for thin-section electron microscopy.¹² Homogenized portions of the eschar were prepared by an established method of negative staining for orthopoxviruses.¹³ The DNA was extracted from approximately 25 µl of Vero-cell cultures infected with a swab of the skin lesion, 50 µg of homogenized eschar, and 25 µg of the homogenized associated skin tissue and was compared with noninfected Vero-cell culture and dilutions of reference vaccinia–rabies glycoprotein virus.^{9,14} Briefly, deproteinization occurred at 37°C for two hours in an extraction buffer containing proteinase K (0.2 mg per milliliter). Purification of DNA was performed by phenol–chloroform extraction. Pelleted DNA was reconstituted in 200 µl TRIS–EDTA buffer (pH 8.0). Amplification of DNA was accomplished with the use of specific primers for the vaccinia virus thymidine kinase gene or the rabies virus glycoprotein gene by denaturation for 2 minutes at 95°C, 40 cycles of denaturation at 95°C for 1 minute, annealing at 55°C for 2 minutes, extension at 72°C for 7 minutes, and final extension at 72°C for 10 minutes. Primer sequences are given in **Table 1**. The polymerase-chain-reaction (PCR) products (20 µl from a 100-µl reaction mixture) were examined by electrophoresis on a 2 percent agarose gel containing ethidium bromide. Specific bands of the expected molecular weight were excised and sequenced with the use of an ABI 377 sequencer (Applied Biosystems, Foster City, Calif.) (**Table 1**). Routine virus-neutralization and indirect immunofluorescent assays were performed to detect antibodies against rabies and vaccinia viruses with the use of serum from the patient and the umbilical-cord blood.^{18,19} Groups of Swiss ICR mice four to six weeks of age were inoculated parenterally and orally with the cell-culture supernatant to evaluate the characteristics of the infectious agent.

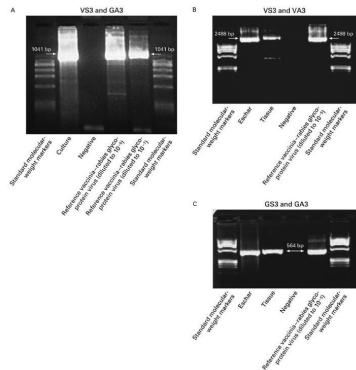
Results

Figure 2.



Electron Micrograph of a Vero-Cell Culture, Taken 48 Hours after Inoculation with Material from a Swab of the Patient's Skin Lesion, That Displays Multiple Stages of Typical Poxvirus Maturation.

Figure 3.



Amplification of Viral DNA in Samples from the Patient.

SUPPLEMENTARY APPENDIX 1. PROTECTION OF MICE FROM LETHAL RABIES-VIRUS CHALLENGE.*				
GROUP	ROUTE	VOLUME	SCHEDULE	NO. OF SURVIVORS/TOTAL NO.
		μl		
1	IM	100	Day 0	9/10
2	IM	100	Days 0 and 7	10/10
3	IP	100	Day 0	9/10
4	IP	100	Days 0 and 7	9/10
5	PO	50	Day 0	4/10
6	ID†	25	Day 0	7/10
7	ID†	25	Day 0	1/5
8	IM	100	Day 0	3/10

*Flasks of Vero cells were inoculated with samples from the original swab of the patient's lesions and incubated for 48 hours. Groups 1 through 6 of Swiss ICR mice four to six weeks of age were inoculated with the indicated volumes of cell-culture supernatant by intramuscular (IM), intraperitoneal (IP), or ID† (ID, oral (PO) routes. Control mice (groups 7 and 8) received phosphate-buffered saline. All of the inoculated mice were challenged with 50 μl of canine rabies-virus var. (3374L, 10^{4.7} median mouse intracerebral lethal doses per milliliter) in the right gastrocnemius muscle. The presence of rabies was confirmed by the direct fluorescent-antibody test on brain tissue.

†A bifurcated needle was used to apply the cell-culture supernatant (group 6) or phosphate-buffered saline (group 7) to five sites on the dorsal surface of the tail.

Supplementary Appendix 1. Protection of Mice from Lethal Rabies-Virus Challenge.

Cytopathic effects were observed in cell culture within 12 hours after incubation with material from the patient. Typical morphologic features of orthopoxviruses were observed by electron microscopy (Figure 2). Positive PCR products were obtained (Figure 3) for all but the samples from the placenta. Sequences exhibited 100 percent homology with those expected for vaccinia–rabies glycoprotein virus (Table 1). Negative control human serum had a titer of less than 1:5 in all tests. In contrast, the titers of antibodies against vaccinia virus in the patient's serum at three weeks after the bite were greater than 1:125 on the virus-neutralization assay and greater than 1:625 on the indirect immunofluorescent assay; the titer of antibodies against rabies virus was greater than 1:18,000 on the virus-neutralization assay. Serum obtained from umbilical-cord blood had a titer

of 1:250 for antibodies against rabies on the virus-neutralization assay. Safety and efficacy data were consistent with the results of previous studies of vaccinia–rabies glycoprotein virus infection in mice (data available as *Supplementary Appendix 1* with the full text of this article at <http://www.nejm.org>).^{3,9}

Discussion

We have documented human infection with vaccinia–rabies glycoprotein virus. For more than a decade, tens of millions of baits containing vaccine have been used throughout Europe and North America. Use of the oral rabies vaccine for wildlife substantially augments conventional programs for the control of rabies. With this approach, western Europe has nearly eliminated rabies in foxes. Nevertheless, the vaccinia–rabies glycoprotein virus is a self-replicating agent, albeit a highly attenuated one, and may cause adverse events, particularly in hosts with altered immunocompetence and in persons for whom smallpox vaccination is contraindicated, such as pregnant women or patients with an exfoliative skin condition.²⁰ Vaccinia immune globulin may be useful if administered promptly for the treatment of eczema vaccinatum and some cases of progressive, ocular, or severe generalized vaccinia, but its effectiveness is questionable if administration is delayed until well after the onset of symptoms.

Despite the large number of baits containing vaccinia–rabies glycoprotein virus that are widely distributed in the field, human contact with the vaccine and adverse events appear to be rare. In northeastern Ohio, from the spring of 1997 to the fall of 2000, 3.6 million baits were deployed over an area of 6497 km² (2509 mi²). Each year, bait was distributed for approximately one week during both spring and fall. In rural areas, aircraft dropped approximately 85 percent of the baits, flying over uniform grid lines 0.5 km (0.3 mi) apart. The remaining 15 percent were delivered by automobile and by hand in urban and suburban areas. Ground-distribution personnel placed baits in areas where raccoons were likely to be found. The target density was 75 baits per square kilometer, or 1 per 3.3 acres. No cases of rabies in raccoons were reported in Ohio during 2000 — a benefit unprecedented among affected regions of the United States over the past 50 years.

With large-scale programs for oral rabies vaccination, people will inevitably find vaccine-laden baits. In Ohio, toll-free telephone numbers are printed on baits, and callers are automatically routed to a rabies information line. Since the inception of the program, 160 reports of human contact with baits have been filed. In 100 reports (62 percent), baits were found in yards and near residences. Twelve reports (8 percent) indicated that baits were found in public areas (parks, roads,

or sidewalks). In the remaining reports, the location of the bait was unspecified. A total of 100 callers indicated that they had had contact only with the outer portion of the bait, with the vaccine sachet intact. In 20 instances, persons had probably been exposed to vaccine, with evidence that the inner sachet had ruptured, usually contaminating the hands.

Three of 160 reports involved persons who listed a health condition that may have been a contraindication to vaccination with the vaccinia virus: one subject was six months pregnant but stated that she had washed her hands after touching the bait; another stated that she had diabetes and rheumatoid arthritis, but follow-up investigations indicated that although she had touched the bait, the vaccine sachet was intact; the third was the patient in the current report. After exposure to the vaccine, the risk of an adverse outcome can be minimized if proper and prompt first-aid procedures, such as hand washing, are followed. Although the overall numbers in Ohio remained fairly consistent each year, reports logged decreased from 7.1 per 100,000 baits in 1997 to 2.1 per 100,000 baits in 2000. Rates of human contact with baits are probably somewhat higher because some incidents go unreported.

Active surveillance of the telephone logs indicates that pets can facilitate human exposure to rabies vaccine. Dogs are attracted to baits and bring them to their owners. Moreover, dogs can rupture the sachet, increasing the chance of human exposure to vaccine. Seventy-eight percent of the reports in Ohio involved domestic animals finding or eating baits. Of these, 120 mentioned dogs, 3 cats, 1 both a dog and a cat, and 1 the possible exposure of cattle at a feedlot. Ten callers described removal of a bait from a dog's mouth. Of 20 reports of contact of the skin with a ruptured sachet, 18 involved dogs. No incidents were documented in which dogs consumed bait and subsequently became ill.

The incident we describe reinforces the message that public and professional awareness is critical to any successful vaccination program. To prevent complications, state programs require ongoing training in the strategic placement of baits to minimize contact by humans and pets. Before bait is distributed, press releases and information describing dates and areas of operation should be given to the media, licensed veterinarians, and infectious-disease specialists in hospitals in the targeted counties. People should be requested to keep pets indoors or confined during baiting campaigns and should be advised to avoid touching baits. Residents should call a toll-free number with questions or to report finding baits. When owners discover a pet eating a bait, they should not risk being bitten by removing it from the animal's mouth. If contact with the vaccine occurs, exposed areas should be washed thoroughly. If signs or symptoms suggestive of infection with vaccinia-rabies glycoprotein virus develop, patients should seek medical consultation. Unfortunately, as oral-vaccination programs become more common, the public and medical

professionals may become more complacent and less likely to seek information or to respond to events that involve exposure to the live recombinant vaccinia–rabies glycoprotein virus.

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